



# **Chemical markers of human waste contamination in source waters: A simplified analytical approach**

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Office of Research & Development

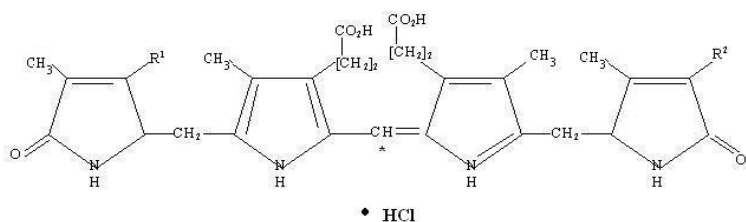
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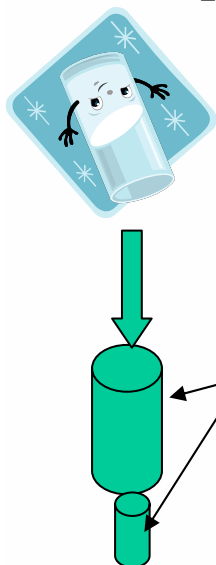
# Introduction

- Giving public water authorities a tool to monitor and measure levels of human waste contamination of waters simply and rapidly would enhance public protection.
- Chemicals shed in feces and urine might be used to detect human waste contamination of environmental waters.
  - Sterols
  - Bile acids
  - Urobilin
    - Finding human-use drugs affiliated with urobilin can help define the waste as human.
      - Azithromycin (antibiotic) and methamphetamine (substance of abuse) were detected



# Experimental

- Samples
  - Grab samples collected, 1L or less, keep cool, above freezing but  $< 4^{\circ}\text{C}$  until extraction w/in 24-hrs
  - pH adjust to  $< 3.0$  with 12N HCl
- Solid phase extraction
  - OASIS HLB cartridges [Waters Corporation (Milford, MA)]
    - 6-mL capacity, 0.2 g, 30- $\mu\text{m}$
    - Prep cartridges 5 mL methanol followed by 5 mL DI water at a rate of 1 mL/min
    - Load samples, 500 mL, into 60-mL reservoirs (60 mLs at a time); start extractions w/ pump at a rate of 4 mL/min
    - Dry samples, via pump, for  $< 5\text{min}$ .
    - Extract with 40 mL methanol:1%acetic acid at a rate of 1mL/min.
    - Nitrogen blow-down extract to 0.5 mL – ready for LC/MS analysis.



# Experimental con't

- $\mu$ LC-electrospray-ion trap mass spectrometer (ThermoQuest Finnigan LCQ™)

## HPLC

- C18 RP, 5  $\mu$ m particle size, 150  $\times$  3.2 mm liquid chromatography column
  - flow rate of 0.40 mL min<sup>-1</sup>, and a 40:60 split after the column, such that 40% of the flow (160  $\mu$ L min<sup>-1</sup>) goes to the ES-ITMS
- Mobile phase: A: 99% water/1 mM ammonium acetate/0.1% acetic acid/1% methanol;  
B: 98% methanol/1 mM ammonium acetate/0.1% acetic acid/2% water.  
100% mobile phase A (hold for 1 min) to 100% mobile phase B (hold for 5 min)  
over a 20-min gradient, with a 5-min equilibrium between runs.



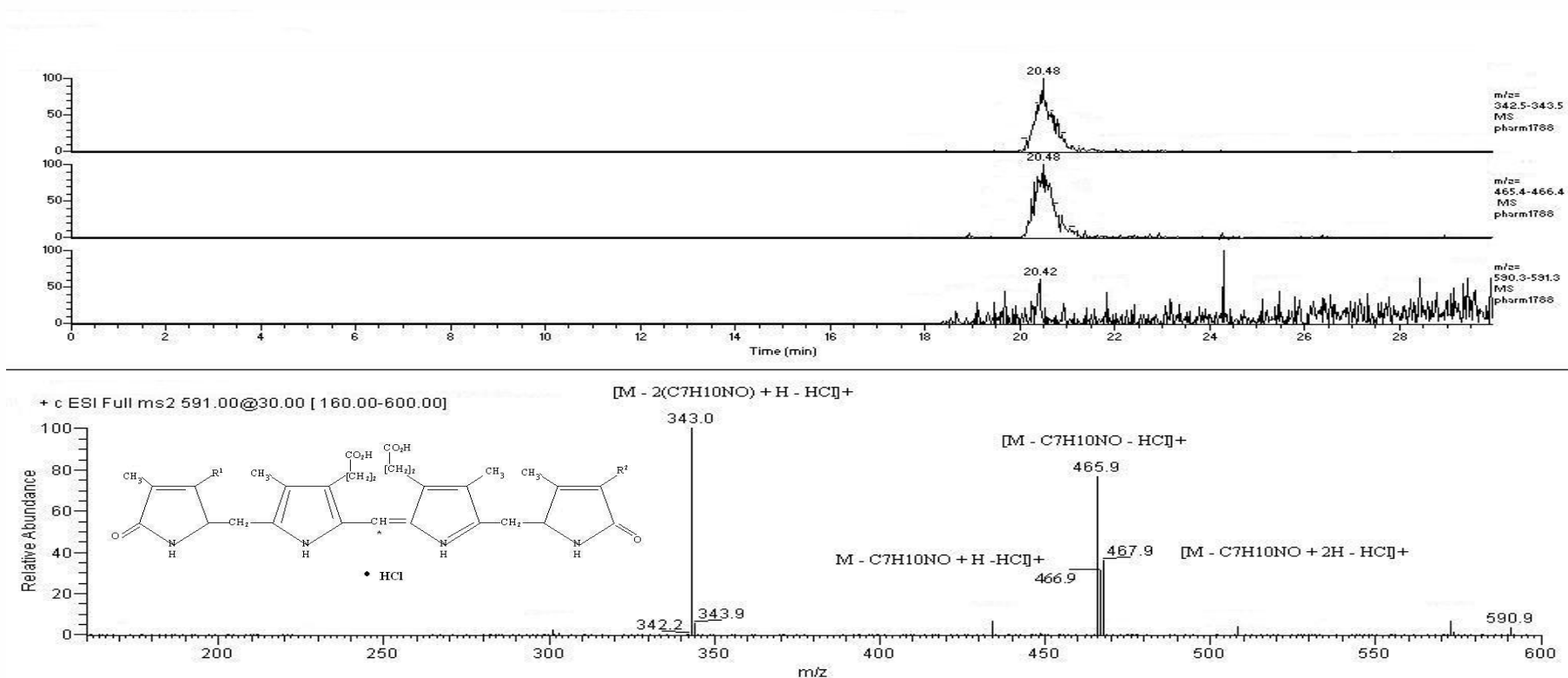
## ES-ITMS

- positive ionization mode
- Screening - scanned from 120 to 830 amu (full-scan mode)
- Heated capillary - 215°C
- For quantifying and confirming - Two other modes, selected ion monitoring (SIM) and collision-induced dissociation (CID), were used.

# Experimental cont

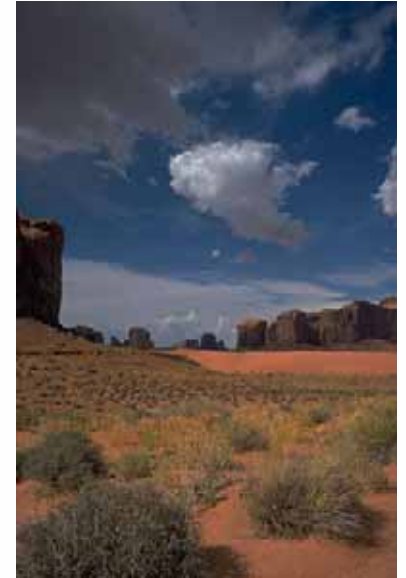
**Limits-of-detection** - Using regression analysis on the data obtained from analyzing urobilin at four different concentrations, using full scan mode, the LOD for urobilin was calculated as 32 pg ( $r^2 = 0.999$ ) on-column.

**CID** – Collision energy = 30%



# Sampling Sites

- Southwest – 1 site in Southern Nevada
- Great Lakes – 2 sites on Lake Michigan



- New England – 18 sites
  - 9 in Maine
  - 9 in Connecticut

## Southern Nevada – 1 site



# Great Lakes – 2 sites

*Lake Michigan beach site 1 (Silver Beach)*

*Lake Michigan beach site 2 (Washington Beach)*

Sample	Urobilin ng/L	Azithromycin ng/L	n
Lake Michigan beach site 1 (Silver Beach)			
June 29, 2004	nd	nd	3
July 13, 2004	nd	nd	2
July 27, 2004	nd	nd	2
August 17, 2004	nd	nd	2
September 8, 2004	no sample		0
Lake Michigan beach site 2 (Washington Beach)			
June 29, 2004	no sample		0
July 13, 2004	nd	nd	2
July 27, 2004	nd	nd	2
August 17, 2004	nd	nd	2
September 8, 2004	nd	nd	2

## New England – Region 1 Maine

### 9 sites

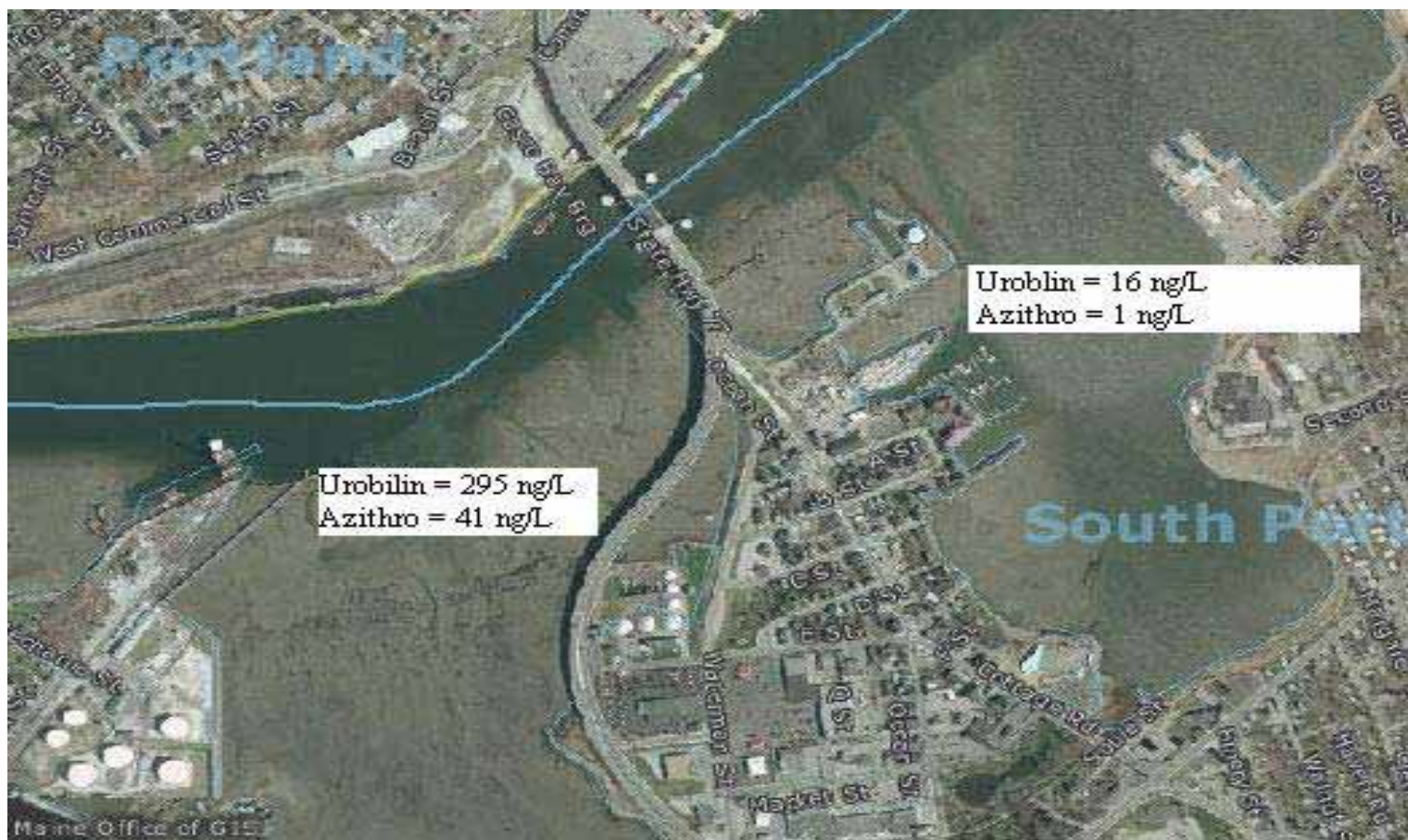
Sample	Urobilin ng/L	Azithromycin ng/L	n
AA26900a – Sanford WWTP	†	77	1
AA26900b laboratory duplicate	†	75	1
AA26901 400 ft downstream Sanford WWTP	33	47	1
<b>AA26902a<sup>2</sup> Yarmouth POTW</b>	<b>11</b>	nd	1
<b>AA26902b laboratory duplicate <sup>2</sup></b>	<b>15</b>	nd	1
<b>AA26903 Royal River Landing</b>	<b>316</b>	†	1
AA26904 Lewiston WWTP	11	nd	1
AA26905 Androscoggin River 0.8 mi downstream Lewiston WWTP	21	†	1
<b>AA26906 South Portland WWTP</b>	<b>295</b>	<b>41</b>	1
<b>AA26907 Fore River - marina</b>	<b>16</b>	<b>†</b>	1
<b>AA26909 Hampden boat landing</b>	<b>52</b>	<b>4</b>	1

nd = non-detect; † positive MS/MS identification, but below LOQ; <sup>2</sup> Methamphetamine detected: 5 ng/L.

## S. Portland Maine WWTP & Fore River Marina

AA26906

AA26907



## Yarmouth Boat Landing AA26903

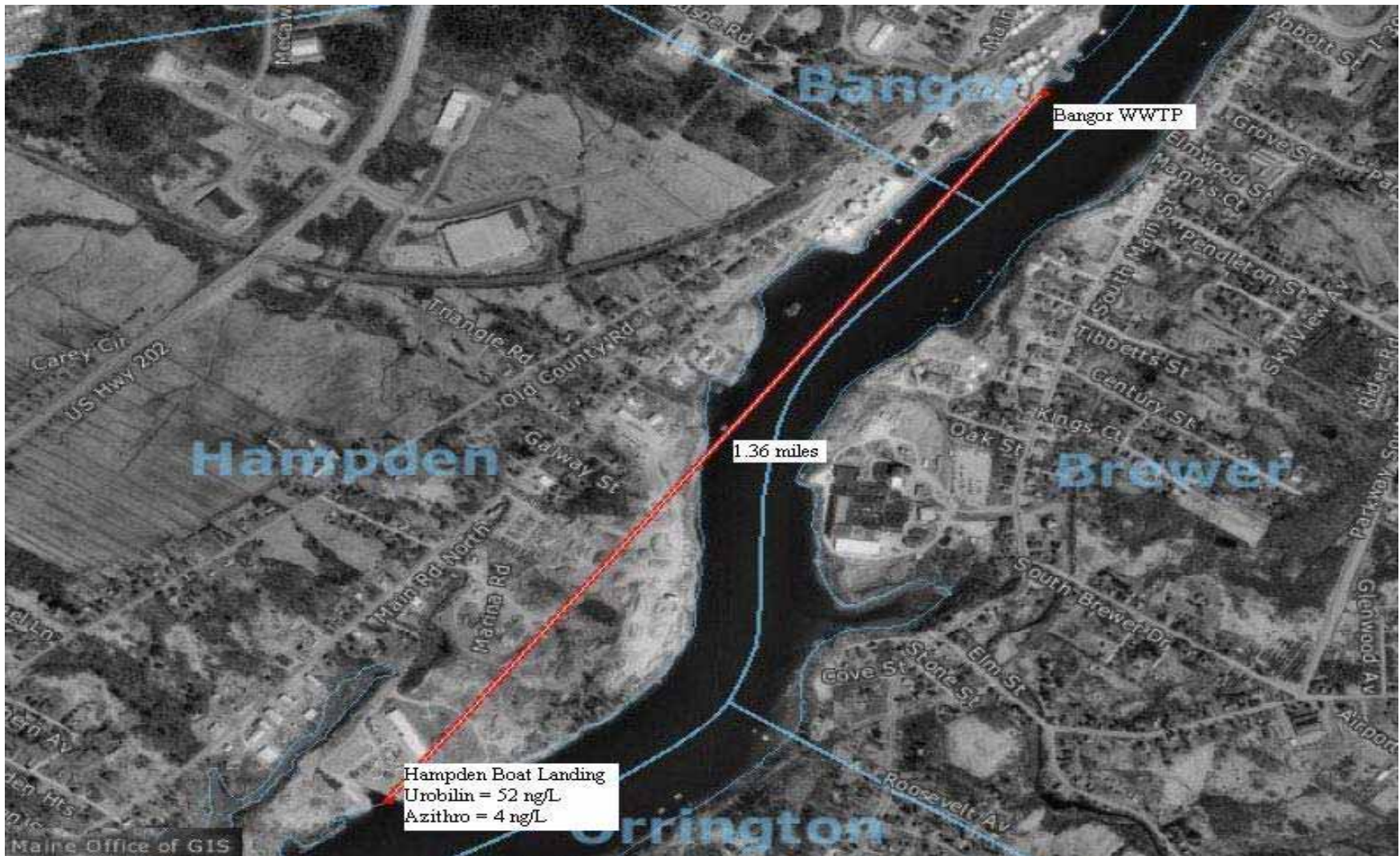
## & Yarmouth POTW AA26902



NOTE: Yarmouth water supply tested positive for coliform Aug and Oct 2002.

Samples were collected Nov 2002. Royal River Yarmouth Town Landing same area.

# Hampden Boat Landing



## New England – Region 1 Connecticut 9 sites

Sample	Urobilin ng/L	Azithromycin ng/L	n
AA29823 – 1.64 miles downstream Manchester WWTP	†	13	1
AA29824 – rural stream no housing in the immediate area	nd	nd	1
AA29825 – densely populated 60% stream flow wastewater 1.25mi upstream	16	5	1
AA29826 – stream flow 70% wastewater	nd	39	1
AA29827 -- stream flow 90% wastewater	†	34	1
AA29828 -- collected 0.8 km downstream from a senior housing condominium complex, which has its own small WWTP	nd	2	1
AA29829 0.65 mi from WWTP	nd	15	1
AA29830 field duplicate of 29829 but collected 15 min apart	42	23	1
AA29831 – 2mi downstream WWTP – failed septic systems 90% wastewater	17	nd	1
AA29832 – 2mi downstream WWTP – stream flow 40 % wastewater	22	nd	1
Control blank	nd	nd	1

nd = non-detect; † positive MS/MS identification, but below LOQ

•None of the Connecticut samples were collected directly from WWTPs. Most were located 1 km or greater from WWTP sewage outfalls, yet both urobilin and azithromycin were detected in some samples.

## Conclusions

- Detection of urobilin, along w/ a human-use drugs can equivocally show that human waste is entering a water source.
- Tertiary/secondary treatment does not seem to efficiently remove azithromycin, as evidenced by its detection at both the Sanford and Portland WWTPs, as they both have similar environmental loadings, 0.5 kg/yr and 0.6 kg/yr

## Future Research

- Investigate correlations between urobilin, nitrate, and coliform levels using principal component analysis

## Acknowledgments

Dr. Y Miyabara, Shinshu University - Education and Research Center for Inlandwater Environment, for his very thoughtful discussions regarding urobilin as a human waste marker; Dr. Jaci Batista, University of Nevada-Las Vegas for sharing Southern Nevada site information; Mr. Peter Philbrook, USEPA Region 1, for sharing his environmental extracts; and Ms. Elizabeth Sams, USEPA ORD/NHEERL, for sampling throughout the summer 2004, at Lake Michigan.

## NOTICE

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